The 3Rs and Animal Welfare – Conflict or the Way Forward?

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Content

Summary

The animal experiment is central to the 3R concept. In European law, animal experiments are classed according to their aims. In the German Animal Welfare Act, they are classed, e.g. as interventions and treatments for experimental purposes, for further education and training, or for the production, preparation, storage or multiplication of substances, products or organisms and for the fulfilment of legal requirements, and are thus regulated with varying strictness. In contrast, in Switzerland all such measures performed on live animals underlie the same approval requirements.

For animal welfarists, the term "animal experiment" includes every intervention and every treatment which is associated with pain, fear and/or suffering and does not directly benefit the respective animal. In the animal experiment, the animal concerned usually suffers as a human would, independent of the experimental goal. Expecting an animal to suffer a treatment one would not want to undergo oneself cannot be in accord with an ethic of respect for fellow creatures. Animal welfarists aim to save animals such suffering. Consequently, they demand the immediate abolition of all animal experiments.

From the perspective of those who allow animal experiments to be performed or who perform them themselves, the goal of the experiment is more important than the animal. Therefore, the following question is central to 3R research: "Can I reach my goal while causing the animal less suffering, using fewer animals or without using animals at all?" The starting point is that the ethical responsibility for man is valued higher than that for the animal. The aim is to protect humans from harm caused by substances and products or from unwanted side effects of medication, to understand diseases and to search for a cure or alleviation of these. When a scientist reaches his goals without using animals, the demand of animal welfarists to abolish the animal experiment is fulfilled.

Zusammenfassung: Die 3R und der Tierschutz - Konflikt oder der Weg nach vorne?

Im Mittelpunkt des 3R-Konzeptes steht der Tierversuch. In der Europäischen Gesetzgebung werden Tierversuche nach Zielsetzung unterschieden, im Deutschen Tierschutzgesetz werden sie bspw. in Eingriffe und Behandlungen zu Versuchszwecken, zur Aus-, Fort- und Weiterbildung und zur Herstellung, Gewinnung, Aufbewahrung oder Vermehrung von Stoffen, Produkten oder Organismen und zur Erfüllung gesetzlicher Vorgaben unterteilt und unterschiedlich streng geregelt. Im Gegensatz dazu unterliegen alle entsprechenden Maßnahmen an lebenden Tieren in der Schweiz dem gleichen Bewilligungsverfahren.

Für Tierschützer umfasst der Begriff „Tierversuch" jeden Eingriff und jede Behandlung, die mit Schmerzen, Angst und/oder Belastung verbunden ist und nicht unmittelbar dem betroffenen Tier dient. Denn im Tierversuch leidet das betreffende Tier unabhängig von seiner Zielsetzung zumeist in einer dem Menschen vergleichbaren Weise. Mit einer Ethik der Mitgeschöpflichkeit ist es nicht in Einklang zu bringen, wenn man einem leidensfähigen Tier das zumutet, vor dem man selbst bewahrt werden möchte. Tierschützer setzen sich daher dafür ein, Tiere vor solchen Leiden zu bewahren. Das bedeutet konsequenterweise, dass sie die Abschaffung der Tierversuche fordern – und zwar sofort.

Diejenigen die Tierversuche zulassen bzw. diese durchführen, sehen nicht in erster Linie das Tier, sondern das Ziel, das es zu erreichen gilt. Im Zentrum der 3R-Forschung steht daher auch die Frage: „Kann ich mein Ziel mit weniger Belastung für das Tier, mit weniger Tieren oder gar ganz ohne Tiere erreichen?" Ausgangspunkt ist dabei, dass die ethische Verantwortung für den Menschen höher eingeschätzt wird als die für das Tier. Es gilt, den Menschen vor Schäden durch Stoffe und Produkte oder unerwünschte Nebenwirkungen von Arzneimitteln zu bewahren, Krankheiten zu verstehen und nach Heilung oder Linderung zu suchen. Da, wo der

These aspects do of course not encompass all the differences and agreements in the positions of animal welfarists and those who support animal experiments. It is not without reason that the discussion around animal experiments is multifaceted and continues to be held intensely from all positions. The classic pattern of a discussion entails that the one side collects the arguments which illustrate that animal experiments lead to wrong results, cannot be transferred and finally stand more against than for the good of mankind. The latter statement is valid, because the wrong methods benefit from investment, thus preventing or hindering new, better research and application of knowledge.

The other side uses, among others, the argument that the person performing the experiment knows the limits of the test procedure and can deal with them accordingly. For further defense of current and future animal experiments, scientists like to present their organ transplant patients, i.e. those who can only lead a life worth living thanks to continuous medication, and admonish that patients with Alzheimer's or Parkinson's disease or with cancer put their last hope into new medical insights which can only be gained with animal experiments.

Animal welfarists argue against this, that the motivation for animal experiments does not always lie in the ethical responsibility for mankind, but also in pure gain of knowledge, titles and money. After all, they also claim that they are protecting man, animal and environment from diseases and want to help them. It is not about saving animals at any price, even including human life, as is commonly brought against them. But they are also not prepared to accept that without real necessity or at any hint of a dilemma, the decision always falls immediately against the animal. The animal experiment may no longer be the method of choice, both for ethical and also for scientific reasons.

What does all of this have to do with 3R research?

It is a fact that animal experiments are still the method of choice – the 'gold standard'. The official German statistics on animal experiments in 2001 (Governmental animal protection report, 2002) counts a total of 2.13 mill. experimental animals, with a tendency towards a further increase. The increase is ascribed to animal experiments especially in basic research, counting 926,294 animals. The other more than a million animals were used for research and development of products and equipment for human, dental or veterinary medicine (509,101), the production or quality control of products or equipment for human, dental or veterinary medicine (289,273), toxicological investigations or other safety tests (189,996), the diagnosis of diseases (26,508), education and training (39 625) and other purposes (145,764).

Before this background, all activities which lead away from animal experiments or reduce the suffering caused in the remaining experimental animals must be judged as positive developments. 3R research leads to a reduction of animal experiments and animal suffering. At the same time, the earnest consideration of the 3Rs also leads to a critical and specific discussion of the animal experiment in question.

Wissenschaftler seine Ziele ohne Tiere erreicht, ist die Forderung der Tierschützer nach Abschaffung des Tierversuchs erfüllt.

Damit allein sind Unterschiede und Gemeinsamkeiten in den Positionen von Tierschützern und denen, die Tierexperimente bejahen, natürlich nicht abgehandelt. Nicht ohne Grund hat die Diskussion um Tierversuche viele Facetten und wird von allen Seiten heftig geführt. Zum klassischen Muster der Diskussion gehört, dass die eine Seite die Argumente zusammenträgt, die verdeutlichen sollen, dass Tierversuche falsche Ergebnisse liefern, nicht übertragbar sind und schließlich auch dem Wohl des Menschen eher entgegenstehen. Letzteres gelte nicht zuletzt, weil in die falschen Methoden investiert wird und so neue, bessere Forschung und Anwendung verhindert oder zumindest behindert werde.

Die andere Seite führt u.a. das Argument ins Feld, dass der Expe-rimentator die Grenzen seines Testverfahrens kennt und damit umgehen kann. Zur weiteren Verteidigung gegenwärtiger und zukünftiger Tierversuche führen Wissenschaftler gerne ihre organtransplantierten Patienten vor, verweisen auf die Menschen, die nur Dank einer Dauermedikation ein lebenswertes Leben führen können und mahnen schließlich an, dass Alzheimer-, Parkinson- und Krebspatienten ihre letzte Hoffnung in neue medizinische Erkenntnisse legen, die nur mit Tierversuchen zu erlangen seien.

Tierschützer halten dagegen, dass die Motivation für Tierversuche keineswegs immer in der ethischen Verantwortung für den Menschen liegt, es gehe auch um wertfreien Erkenntnisgewinn, um Titel und um Geld. Schließlich nehmen sie für sich in Anspruch, dass auch sie Mensch, Tier und Umwelt schützen, vor Krankheiten bewahren und ihnen helfen möchten. Es gehe nicht darum, Tiere um jeden Preis, wenn es sein muss, auch auf Kosten von Menschenleben, zu retten, wie ihnen gerne unterstellt wird. Aber sie sind auch nicht bereit zu akzeptieren, dass gänzlich ohne Not oder bei jeder Andeutung eines Dilemmas die Entscheidung sofort zu Ungunsten des Tieres fällt. Der Tierversuch darf, und zwar sowohl aus ethischen als auch aus wissenschaftlichen Gründen, nicht länger das Mittel der Wahl sein.

Was hat das alles mit der 3R-Forschung zu tun?

Tatsache ist, dass Tierversuche immer noch das Mittel der Wahl – der „goldene Standard" – sind. Die offizielle deutsche Tierversuchsstatistik von 2001 (Tierschutzbericht der Bundesregierung, 2002) weist insgesamt 2,13 Mio. Versuchstiere aus, mit steigender Tendenz. Für den Anstieg der Tierversuche ist insbesondere die Grundlagenforschung mit insgesamt 926.294 Tieren verantwortlich. Die verbleibenden mehr als eine Million Tiere sind der Erforschung und Entwicklung von Produkten und Geräten für die Human-, Zahn- oder Veterinärmedizin (509.101), der Herstellung von oder Quali-tätskontrolle bei Produkten oder Geräten für die Human-, Zahn- oder Veterinärmedizin (289.273), toxikologischen Untersuchungen oder anderen Sicherheitsprüfungen (189.996), der Diagnose von Krankheiten (26.508), der Aus- und Weiterbildung (39.625) und sonstigen Zwecken (145.764) zuzuordnen. Vor diesem Hintergrund müssen alle Aktivitäten, die vom Tierversuch wegführen bzw. den verbleibenden Versuchstieren ihr Schicksal erleichtern, positiv gesehen werden. 3R-Forschung führt zu einer Einschränkung von Tierversuchen und zu einer Verminderung von Tierleiden. Gleichzeitig führt die ernsthafte Auseinandersetzung mit den 3R auch zu einer kritischen und spezifischen Diskussion um den jeweiligen Tierversuch.

Keywords: 3R, replacement, reduction, refinement, animal experiments, animal welfare, Animal Welfare Act, European legislation

1 State of the art

The 3Rs concept is centred on animal experiments. In European legislation, animal tests are categorised according to their respective purposes. For example, in the German Animal Welfare Act, they are subdivided into procedures and treatments for experimental purposes, for the purpose of education and continuing education, and for the production, storage or multiplication of substances, products or organisms, and lastly for the fulfilment of legislative demands. In accordance to their purpose, different legislative rules with differing levels of stringency apply. In contrast, in Switzerland all animal experiments are covered by the same licensing procedure, irrespective of their purpose.

For animal welfarists, the term "animal experiment" covers all procedures and all treatments that inflict pain, fear and/or distress on the animals, provided that they are not performed with the direct aim of helping that specific animal. It is taken for granted that the individual animal suffers from the experiment regardless of its purpose in a manner that is at least similar to the way a human being would suffer under the same circumstances. When truly applying the ethical principle of animals being "co-creatures", it is unacceptable if sensitive animals are forced to endure treatments, which humans themselves are not willing to endure. Animal welfarists stand up for preventing animals from having to endure such suffering. In consequence, this means that they demand the abolition of animal experiments – and this without delay.

Those people who consider animal experiments to be acceptable or who perform them themselves don't see the animal in first instance, but the goal envisaged by the performance of the experiment. Therefore one of the main questions that underlie all research in the 3Rs principle is the question of whether one can achieve one's goal with less distress for the animal, with less animals or even altogether without animals. This attitude is based upon the conviction that the ethical responsibility towards humans has a higher value than the responsibility towards animals. The main incentive is to prevent humans from having to suffer from damages through substances or products or from unwanted side effects through pharmaceuticals, to understand diseases and to strive for their cure or alleviation. Whenever a scientist finds a way to achieve these goals without animals, the animal welfarists' request for the abolition of animal experiments is fulfilled.

Evidently, this explanation does not go far enough to describe the differences and the overlaps of the positions of animal welfarists and of those who accept animal experiments. It is not without reason that the discussion on animal experiments is so multi-facetted and is held with such high emotions. A typical pattern for this discussion would be that the one side presents arguments that show that animal tests lead to incorrect results, that they poorly represent the situation in humans and thus are not beneficial to humans. The latter point also being true, since investments are being made into the wrong methods, which prevents a new and better kind of research and application of test methods from becoming reality (or at least impedes it).

A typical argumentation from the other side is that the experimenter very well knows the limits of his test methods and knows how to cope with them. In further defence of animal experiments that are currently being performed or that are planned in the future, scientists tend to refer to patients that have received organ transplantations or that are receiving life-long medicaments without which they would be unable to lead an acceptable life. And lastly they recollect that patients suffering from Alzheimer's disease, from Parkinson's or from cancer are putting all their hopes into future medical discoveries and that these can only be achieved with animal tests.

On the other side, animal welfarists contend that the motivation to perform an animal experiment does not always lie in the ethically motivated desire to help humans, but also in the desire to achieve neutral knowledge gain, in the desire for financial profit and for obtaining a higher professional degree. They also claim to be motivated by wanting to help and protect humans, animals and the environment, and to prevent them from diseases. They emphasise that there is no reason to believe that they want to save animals at no matter which price – even at the cost of human life – which is often purported by the other side. But they are also not willing to accept that without further questioning or at the slightest hint of a dilemma the decision immediately is made to the animal's disadvantage. Both for ethical and for scientific reasons, it is no longer acceptable if the animal experiment remains the "standard procedure".

2 How are these arguments linked to research in the 3Rs?

It is a fact that animal experiments are still considered to be the method of choice – the "golden standard". Thus scientists tend to make use of those techniques they have become acquainted with during their education or that are used traditionally when pursuing the respective questions. The same is true in the area of safety testing of substances where many representatives of authorities and toxicologists trust in animal tests of poor scientific quality, since they have become accustomed to the consequences of their flaws – the residual risk.

Year after year, in Europe alone, millions of animals have to endure pain, suffering and damage for scientific purposes. Whereas in the first years of official data collection on animal use, the numbers of laboratory animals decreased noticeably year after year, in the last years only a minimal decrease was discernible (Fig. 1).

In those member states of the European Union that compile more or less reliable and complete statistics on laboratory animal use, such as the United Kingdom, the Netherlands or Germany, currently even a tendency towards an increase in animal experiments can be seen. In the United Kingdom, 3.32 million animal experiments were reported in 1989 and over 2.62 million in the year 2001; the official German statistics report over 2.64 million animals for the year 1989 and close to 2.13 million laboratory animals for the year 2001, and in the Netherlands the respective numbers are close to 1 million animals in the year 1990 and close to 0.71 million in the year

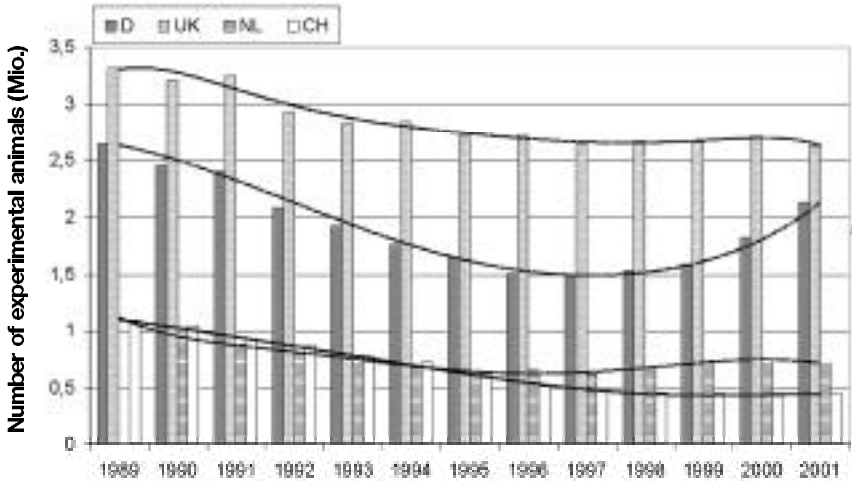

Fig. 1: Number of experimental animals in Germany (D), the United Kingdom (UK), the Netherlands (NL) and Switzerland (CH).
Note: For Germany the number in 2000 and 2001 was recorded according to the new experimental animal registration regulation. For the Netherlands, until 1993 the number of registered animal experiments is represented, and from 1994 onwards the number of experimental animals used.

2001. In Switzerland, the numbers have still decreased year after year, even though this tendency lately has scarcely been noticeable. In the year 2001, 0.45 million animals were reported in the Swiss statistics, whereas the number for 1989 was above 1 million.

Most of the laboratory animals reported in the official statistics were used for the development and testing of pharmaceuticals and other substances. The relative number of animals used in the field of basic research has been increasing lately; the booming area of gene-technology is given as an explanation for this phenomenon. In the year 2001, the United Kingdom reported approximately 30% of the animals used in the field of basic research, in Germany the figure is 44% (2001 was the first year that the numbers were compiled according to the new decree on the reporting of laboratory animal use; the figure was 28% in 1999) and in Switzerland just under 26%. In Germany, 9% of the laboratory animals reported in 2001 were used in toxicological studies (Switzerland 13%, United Kingdom 6%, Netherlands 8%).

Taking into consideration these numbers, all endeavours that lead away from animal tests or that alleviate the fate of the remaining laboratory animals have to be supported. Research on the 3Rs principle leads to a reduction of animal experiments and to a reduction of animal suffering. At the same time, serious efforts in the field of the 3Rs result in a critical and specific discussion on the respective animal experiment. In detail, this implies:

3 Refinement

Refinement deals with the question, if and how the suffering of laboratory animals can be reduced during an experiment or test. As a prerequisite one has to consider the extent to which the animals actually are being distressed in the experiment. It is by no means that this would go without saying – even if one would expect the contrary. For example, Lindl et al. (2001) came to the conclusion that in two thirds of the applications for the licensing of animal experiments they evaluated in their survey, the distress for the animals was underestimated in comparison to an estimation made along the lines of the Swiss Distress Score. Völkel and Labahn (1997) came to similar results. Therefore scientists that deal with the question of how to alleviate the plight of an animal during a specific experiment or treatment or during the post-procedural stage will be more aware of the consequences of their activities and of their underlying responsibility.

Refinement in a broader definition also implies keeping laboratory animals under conditions that enable them to fulfil their basic ethological and physiological needs and thus cause them the least possible distress. This is another area, in which creating awareness is badly needed. Until today, a great number of the laboratory animals used in Europe are kept under conditions that do not stand in line with the state of the art of ethological science. All in all however, it goes without saying that refinement makes no direct contribution to the goal that animal welfarists are striving for, that is to get away from the animal experiments as such as quickly as possible.

4 Reduction and replacement

Reduction deals with trying to find out whether the scientific problem in mind can be answered with fewer animals than originally planned and whether parts of the problem can be examined making use of non-animal test methods. It is also in this area that the relevant discussion goes beyond the concrete question of numbers of animals to be saved. The following questions are also to be asked: “Which answers are sought for with the results of the animal experiment under scrutiny? Will the result truly be more substantial the more animals are used – as a statistical fundament? Might it be possible to come to conclusions with the initial results, so that one can do without the mere tick boxing of parallel animal experiments without losing information?” The ultimate goal of reduction should be to reduce the number of laboratory animals used to an extent that no more animals are being used – that is their total replacement. This means that the respective scientific goal that previously was pursued in an animal experiment will now be pursued with new means – that is with different test methods. It is inevitable that such a task will lead to a critical examination of the respective animal experiment – when

striving for replacement even more so than when striving for reduction. The reasons for this will be discussed further below.

From the point of view of animal welfare, reduction is the first step towards the replacement of animal experiments. Their replacement is the only true and consequent decision in interest of humans and the animals.

5 The establishment of the 3Rs principle

The request to perform research with the fewest number of animals possible and with the minimum of animal suffering possible was first put forward in the 18th century (Maehle, 1992). Russell and Burch published their 3Rs concept in 1959. However, it was only in the beginning of the 1980s, when the controversial discussions surrounding animal experiments, their minimisation and their replacement, were at their high point for example in Germany that the 3Rs principle first led to political measures.

6 The legislative implementation of the 3Rs principle

In the 1980s, the 3Rs principle was first implemented in animal welfare legislation. On European level, requirements on the 3Rs principle were laid down in 1986 both in the European Convention ETS 123 of March 18th, 1986, for the protection of vertebrate animals used for experimental and other scientific purposes and in Council Directive 86/609/EEC of November 24th, 1986, on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. In these documents it is stated: *"An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available"* (Article 6 of the Convention; Article 7(2) of the EU-Directive). The procedure must be chosen which uses *"the minimum number of animals"* (Article 7 of the Convention; Article 7(3) EU-Directive). The procedure must be chosen which causes "*the least pain, suffering, distress or lasting harm"* (Article 7 of the Convention; Article 7(3) of the EU-Directive). Furthermore, in Article

Tab. 1: Examples of institutions that promote the 3Rs in Europe and the USA

1969	**United Kingdom**	Foundation **FRAME** (Fund for the Replacement of Animals in Medical Experiments)
1976	**Switzerland**	**FFVFF** (Fonds für versuchstierfreie Forschung – Fund for Research without Animal Experiments)
1980	**Germany**	Establishment of the research funding programme "replacement methods for animal experiments" by the Federal Ministry of Research and Technology (now Federal Ministry of Education and Research)
1981	**USA**	**CAAT** (Centre for Alternatives to Animal Testing) at the Johns-Hopkins-University in Baltimore
1981	**Germany**	Research award to promote alternative methods (about 15.000.- Euro) by the Federal Ministry of Health (now Federal Ministry of Consumer Protection, Agriculture and Food)
1985	**USA**	International Foundation for Ethical Research
1986	**Germany**	**Akademie für Tierschutz** of the Deutscher Tierschutzbund (Animal Welfare Academy of the German Animal Welfare Federation)
1986	**Germany**	**set** (Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen – Foundation for the Promotion of Research on Replacement and Complementary Methods to Reduce Animal Testing)
1987	**Switzerland**	Foundation 3R (**Stiftung Forschung 3R**)
1987	**The Netherlands**	**National platform on alternatives to animal experimentation**
1989	**Germany**	**ZEBET** (Zentralstelle für die Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch – Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments) of the Federal Ministry of Health (now Ministry of Consumer Protection, Agriculture and Food)
1990	**USA**	**UC ALERT** (UC Centre for Animal Alternatives at the University of California)
1992	**European Union**	**ECVAM** (European Centre for the Validation of Alternative Methods) at the Joint Research Centre in Ispra, Italy
1993	**Austria**	**MEGAT** (Mitteleuropäische Gesellschaft für Alternativmethoden zu Tierversuchen – Middle European Society for Alternative Methods to Animal Experiments)
1994	**USA**	**ICCVAM** (Interagency Coordinating Committee on the Validation of Alternative Methods)
1994	**The Netherlands**	**NCA** (Netherlands Centre for Alternatives to Animal Use) at the University of Utrecht
1996	**Austria**	**zet** (Zentrum für Ersatz- und Ergänzungsmethoden zu Tierversuchen – Centre for Replacement and Complementary Methods to Animal Experiments)
1999	**Belgium**	**BPAM** (Belgian Platform on Alternative Methods)
2001	**Europe**	Process of foundation of **ECOPA** (European Consensus Platform on Alternative Methods)

7(3) of the Directive it is requested that experiments must be chosen that *"involve animals with the lowest degree of neurophysiological sensitivity"*. Article 23(1) of the Directive states: *"The Commission and Member States should encourage research into the development and validation of alternative techniques which could provide the same level of information as that obtained in experiments using animals but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field."*

The revised version of the German Animal Welfare Act dating from August 18th, 1986, was the first version, in which it was requested that when deciding whether certain animal experiments are inevitable (that is experiments according to the definition of the Act as well as procedures and treatments performed in student and continuing education), one had to examine if the goal pursued could not be achieved with other methods or procedures. The authorities responsible for the licensing of animal experiments were backed up with advisory committees. In § 9 of the German Animal Welfare Act, further regulations were introduced that oblige the experimenter to reduce the distress for the animals. However in Germany it was only in 1998 that these regulations became applicable to procedures and treatments on animals for the production and storage of substances, products or organisms.

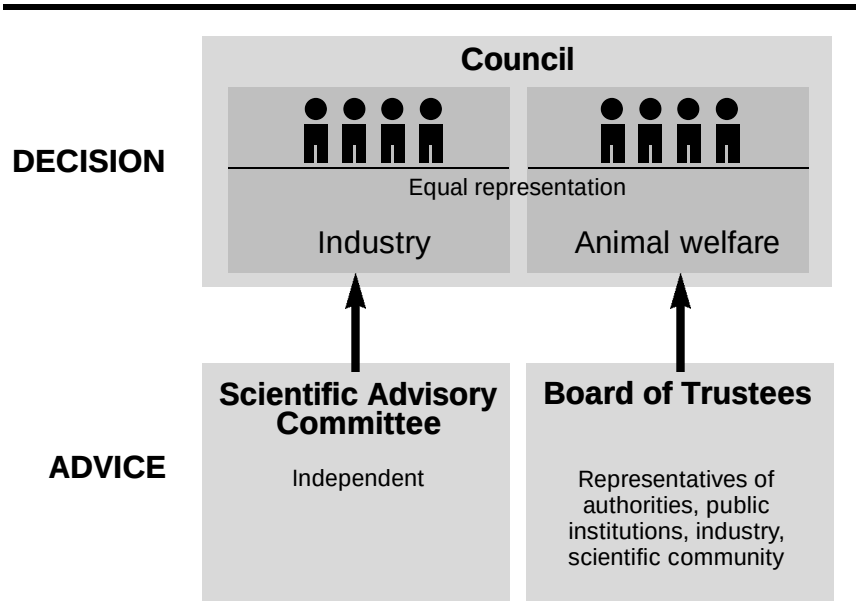

Fig. 2: Structure of the German foundation set (Foundation for the Promotion of Research on Replacement and Complementary Methods to Reduce Animal Testing).

7 Institutions active in the area of the 3Rs principle

The two official institutions ZEBET (the National German Centre for Documentation and Evaluation of Alternatives to Testing in Animals) and ECVAM (the European Centre for the Validation of Alternative Methods) play a predominant role in the area of the development and acceptance of alternative methods in the field of the testing of substances. It was in 1989 that in Germany the joint requests of the former Federal Association of the Pharmaceutical Industry and the animal welfare movement were met and ZEBET was founded as an official authority at the former Federal Health Agency (now: Federal Institute for Risk Assessment). ZEBET's mission is to strive for the reduction and replacement of animal experiments. In 1992, ECVAM was founded by the European Commission at the Joint Research Centre of the European Union in Ispra, Italy. It is to be welcomed that in 1994 such an authority also was installed by the Federal Government of the United States, i.e. ICCVAM, the Interagency Co-ordinating Committee on the Validation of Alternative Methods. This Committee is responsible for initiating and co-ordinating the acceptance of alternative methods on behalf of relevant American Authorities (Tab. 1).

There is yet another initiative that strives for the promotion of the 3Rs and that has proven successful in Europe. The German Foundation set (the Foundation for the Promotion of Research on Replacement and Complementary Methods to Reduce Animal Testing) has been in existence for 15 years. This Foundation was established in 1986 in the course of the revision of the German Animal Welfare Act on the initiative of the Federal Minister for Nutrition, Agriculture and Forestry, who is responsible for animal welfare in Germany. In this context, one of the main driving forces was that the topic of "animal experiments" stood in the centre of the discussions and of public attention during 1986. Amongst other issues, it was deplored that the development and promotion of new non-animal test methods was too slow and too inefficient.

The Foundation was intended to be a new platform at which the interest groups involved were to make a common effort to promote the further development of methods for the replacement and supplementation of animal experiments – regardless of their background and differing basic principles. It was intended to promote the dialog on animal experiments and alternative methods, to establish transparency on relevant processes and to open up possibilities to try out unconventional strategies aimed at reaching the goal more quickly. The Foundation's representatives are the Association of the Chemical Industry (Verband der Chemischen Industrie), the Association of the Researching Pharmaceutical Producers (Verband forschender Arzneimittelhersteller), the Industrial Association for Body Care and Detergents (Industrieverband Körperpflege und Waschmittel) and

the Industrial Association for Agricultural Products (Industrieverband Agrar), on the one side, and the largest national animal welfare organisations (Bundesverband Tierschutz, the Federal Association for Animal Welfare, and the Deutscher Tierschutzbund, the German Animal Welfare Federation), on the other side. The Foundation's board of trustees is formed of representatives of the Federal and State Ministries as well as of the Trade Unions and of the Churches. From the very beginning, the efficient development of the Foundation has been based upon the fact that in the Executive Committee responsible for all decisions the animal welfare movement and the industry were equally represented, and this is still true today. The Foundation contributes to the funding, further development and adoption of alternative methods and to the prevention of animal experiments. The financial support for these activities is granted mainly by the industry (Fig. 2).

Similar foundations were established in the Netherlands and in Switzerland in 1987, in Austria in 1996, and in Belgium and in Spain in 1999. On the international level, efforts are currently under way to join together the national platforms to a powerful European lobbying force for the promotion of alternative methods, the European Consensus Platform for Alternative Methods (ecopa).

8 The practical application of the 3Rs principle

In the following, some concrete examples are given to demonstrate the outcome of all these initiatives and of the legislative request to the researchers to refine, reduce and replace animal experiments. These examples shall also point out the obstacles that had to be overcome and still remain to be overcome when pushing for the acceptance of alternative methods.

8.1 Examples for the 3Rs in the area of legally required animal experiments

This is the area of animal research in which the results achieved in terms of the 3Rs are best discernible, since this is an area in which the goal of replacing, reducing and refining animal tests aims at concrete, precisely defined experiments, that is those tests that are performed for the determination of specific endpoints. At the same time, this is an area in which special problems arise, since the respective alternative methods have to undergo an extremely strict scientific evaluation of their relevance and reproducibility, the so-called validation, and then also require lengthy bureaucratic processes for the inclusion of the test methods into the legal testing regimes.

8.2 Refinement

8.2.1 Example No. 1: The Local Lymph Node Assay (LLNA)

The evaluation of allergic skin reactions is a good example for the development of a refinement test method. The traditional methods for testing skin sensitisation are the "Guinea Pig Maximisation Test" and the "Occluded Patch Test", during both of which the test substance is applied onto the skin of guinea pigs and the resulting pathological changes are recorded by subjective evaluation. The new, refined method is the LLNA, the Local Lymph Node Assay (Huggins, Chapter 4.2). The LLNA has been accepted to be scientifically valid for the testing of skin sensitisation by the Scientific Advisory Committee of ECVAM (Balls and Hellsten, 2000) and was adopted as an OECD test guideline in 2001 (Huggins, Chapter 4.3).

8.2.2 Example No. 2: Quality control of rabies vaccines

The area of quality control of vaccine batches encompasses tests that are very distressing to the laboratory animals. For example, in the efficacy testing of rabies vaccines, a minimum of 120 mice is required for the testing of each single batch (Halder, *ALTEX* Suppl. 01/2001). For the determination of whether the batch is able to induce sound protection from rabies infections, several groups of animals are vaccinated with different concentrations of the test batch. Afterwards the animals are infected with the rabies virus. The control group that has not been vaccinated is also infected. Thus, all the animals of the control group will develop rabies; the vaccinated groups will develop the symptoms to differing extents. The quality of the vaccine is determined according to the number of animals of each group that die or show symptoms of rabies after a certain time period.

An important challenge in the area of refinement is to determine cut-off criteria for distressing experiments in order to save the animals from having to suffer unnecessarily. By combining measurements of the decreasing body temperature with observations of the first neurological disorders that occur at the onset of the disease – the movements of the animals become slower and they turn in circles, the tests for the quality control of rabies vaccines can be cut off three to five days before the animals would die without affecting the test results (Cussler et al., 1998; Hartinger et al., 2001). The animals are thus saved from having to endure progressing symptoms of the disease such as severe weight loss, rise in body temperature and paralysis.

8.3 Conclusions

The LLNA is a good example that shows that refinement methods are not only beneficial from the point of view of animal welfare but that they also have scientific and economic advantages. The level of consumer protection is improved, when such methods come into use.

In order to save the animals from having to endure unnecessary pain and distress, it is necessary to stop animal experiments at the right time. On the one hand, the tests may not be stopped before the effects of the test substance can be determined; on the other hand, moribund animals have to be killed without delay in order to reduce their distress as far as possible. In order to find a balance between these two sides, diligent observations are necessary to enable the determination of the first external clinical symptoms the animals develop; otherwise the test cannot be cut off at the right time. Therefore the care personnel not only have to be trained adequately but it must also be ensured that the time required for the observations is available every day of the week. However such measures are not necessarily provided voluntarily for a routine test.

From the point of view of animal welfare, the choice of humane endpoints for

efficacy and toxicity testing should go without saying. This is also an official request, for example laid down in the German Animal Welfare Act, in which it is said that animal experiments must be stopped and the animals released from their suffering as soon as it is discernible that the experiment will lead to the death of the animals. However in reality there are many test guidelines in which death is still laid down as the endpoint of the method. As long as there are no specific compulsory cut-off criteria for all of these tests, it depends upon the sensitivity and the commitment of the experimenter and the care personnel when a test is stopped.

In addition, for all severe experiments with animals it should be considered whether it is possible to provide analgesics or anaesthetics during the procedure and whether the protocol could be changed to make the experiment more endurable for the animals. Such measures should also be adopted into the official guidelines.

8.4 Reduction

8.4.1 Example No. 1: Acute oral toxicity testing/the LD_{50}

The acute toxicity testing is a typical example that shows that the critical analysis of a test method can lead to a considerable reduction of the number of animals used without affecting the meaningfulness of the test. The determination of the acute toxicity of a substance serves to classify the substance according to its degree of toxicity. Therefore mainly notification authorities were interested in labelling all substances with a concrete numeral value that was to characterise its toxicity. This led to the development of the so-called LD_{50} test, during which the concentration of the test substance that leads to the death of 50% of the animals is determined. The purpose of the test was thus to determine a statistical number. Therefore it required a certain amount of animals upon which to base the calculations (for more detailed descriptions, see Huggins, Chapter 1).

In the meantime three alternative test methods (the Acute-Toxic-Class-Method, the Fixed-Dose-Procedure, the Up-and-Down-Method) have been developed, all of which require fewer animals (6-10 instead of more than 40 animals), and the suffering of the animals has been reduced (see also the contribution of Huggins, Chapter 1). All three methods have been adopted as OECD test guidelines as officially accepted test methods for the determination of acute oral toxicity.

Already early on, the LD_{50} test was heavily criticised, because of its doubtful scientific value, its poor reliability and last but not least because of the severe distress and suffering that is inflicted upon the animals used. It was already in 1964 that Griffith pointed out that the LD_{50} value of chemicals can vary depending on the species, the line, the age, the sex, the nutrition status and the housing conditions of the animals used. Even when the same substance is tested in the same species in different laboratories, the results of the LD_{50} tests can differ between three- and eleven fold (Hunter et al., 1979).

The largest problem with regard to the current state-of-the-art is that in spite of the acceptance of three reduction methods, the LD_{50} test remained an accepted test method for much too long. Therefore it kept being performed, and European authorities accepted the data gained with it. In Germany, the responsible Federal Ministry acknowledged that in the year 2000 10% of the evaluations of acute oral toxicity of chemicals were still performed with the classical LD_{50} Test. To overcome this problem, additional efforts were made to remove the LD_{50} test from the OECD test guidelines. After endless years of discussions, this test was finally removed from the OECD test guidelines at the end of 2001.

8.4.2 Example No. 2: The determination of the wastewater fee with the fish test

The German Wastewater Act requires companies that introduce wastewater into public waters to perform a fish test in order to determine the fee they have to pay for the introduction of this wastewater. For this purpose, fish are put into different concentrations of the wastewater and the concentration is determined at which no fish dies. For many years, the reliability and reproducibility of a fish cell test was evaluated to determine its meaningfulness as an alternative to the fish test. The mechanism of this fish cell test is based upon the fact that damaged cells can no longer incorporate a specific dye. The amount of dye not absorbed by the cells can be determined photometrically. The data of the mentioned validation study were of the highest scientific standard; however the cell test did not lead to the same results as the fish test. This impeded the official acceptance of the fish cell test. However due to the increasing pressure, authorities and industry considerably reduced the number of fish requested for the testing of the wastewater samples.

In the end, the replacement of the fish test that is used in fulfilment of the Wastewater Act failed, because it was not possible to find a test method that leads to the exact same results as the animal test. The animal species prescribed for the evaluation of fish toxicity is the golden ide. However when comparing the LC_{50} values for different chemicals in the golden ide in the literature, the values reported in different publications differ considerably for many chemicals. In addition, different fish species have differing levels of sensitivity to the chemicals (Juhnke et al., 1978; Amann, 1989). Thus the attempt to exchange the golden ide as animal species to a different fish species also would be prone to failure, if one expects the substitute to lead to the same results.

However up until today there is no willingness to grant the system for the calculation of a wastewater fee a fundamental change – after all, one has to keep in mind that the purpose of this test exclusively deals with the determination of a monetary fee and not the protection of the environment or human safety. Up until today there is no willingness to regard fish as sentient creatures. Those responsible stick to the test system chosen, even though there is no reason why the choice originally fell onto this system – especially when defenders of the fish test pretend that the goal is not only money but also the protection of ecosystems. Disregarding the fact that fish are only part of the aquatic habitat, an arbitrarily chosen fish species is also not representative. In spite of all this, only the number of golden ides to be

used has been changed– it seems as if the burden on the companies through the wastewater fee may not to be affected. All in all, it is evident that this is yet another case where the endeavours to find a replacement alternative got stuck in the course of the reduction phase. In the beginning of the year 2001, a German National Standard (DIN 38415-T6) was compiled for a test with fish embryos. The fish egg test was included in the waste water regulations in the summer of 2002, however only in addition to the animal experiment. The fish test still had to be performed in certain cases. It is only in autum 2003 that the complete replacement of the fish test is finally being initiated: The Federal Ministry for the Environment has put forward drafts of the waste water regulations and the waste water levy act, in which the fish test is being omitted.

8.5 Conclusions

It is totally unacceptable when in spite of the existence of accepted alternatives, it remains possible to perform the test method that inflicts more distress on the animals and uses more animals. To overcome this problem, it is necessary to remove the "old" animal test method from the test guidelines as soon as an alternative that leads to the same level of information has been officially accepted.

Whereas up until today animal test methods will be accepted by official authorities and included into legal requirements without in-depth evaluation, much more stringent measures are applied to non-animal test methods (Kolar, 1998). And this is true even though they are far better than the animal tests in terms of reliability, reproducibility and independence from a specific laboratory. In spite of this their official acceptance will fail if their results differ from those of the animal tests to be replaced.

8.6 Replacement

8.6.1 Example No. 1: Phototoxicity

It is now possible to test phototoxic effects of chemicals without using animals. The evaluation of the potential of substances to become toxic when exposed to UV light can be done in a simple cell culture test. In this test permanent cell cultures are exposed to the test substance and UV light simultaneously. As an endpoint it is measured whether the resulting reaction impedes the inclusion of a dye into the cells. On June 8th, 2000, this test was published in the Official Journal of the European Union (Directive 2000/33/EC). It replaces a test with guinea pigs or rabbits, during which the test substances are applied onto the shaved skin of the animals before these are fixated and exposed to UV radiation. As a result, the animals can suffer from damages such as skin irritation and even skin destruction or severe burns.

The cell culture test for the determination of phototoxic effects is the first non-animal test method that was scientifically evaluated according to an internationally accepted procedure and that in the meantime has at least been accepted on the level of the European Union. However this successful method almost failed during its validation phase (Spielmann et al., 1995). The reason for this was that the validity of an alternative method is generally evaluated against the results from animal tests. In a joint EU/COLIPA study (COLIPA: The European Cosmetic Toiletry and Perfumery Association) for the validation of *in vitro* test methods on phototoxicity, the test substance Piroxicam was classified to be phototoxic according to the test data from animal tests. In the *in vitro* test, no phototoxicity could be determined; therefore this *in vitro* test result was considered to be false negative. Since it is much worse if a new test method cannot recognise a potential hazard than if it recognises a hazard that doesn't exist, the chances were low that the *in vitro* test would ever become accepted. In the end however, human data showed that the *in vitro* test result was correct and the animal test result was false. Unfortunately, data on effects in humans are often not publicly available. However they would be badly needed in order to determine which results of which test methods lead to the best predictions of possible hazards to humans.

In 1997, after seven years of validation, the cell culture test for the determination of phototoxic effects was declared successfully validated by the ECVAM Scientific Advisory Committee. Afterward it took three more years, until, in June 2000, the method was published (2000/33/EEC) in Annex V of the Directive on the classification of dangerous substances (67/548/EEC) after which it became legally binding in the European Union. After a further delay, in November 2001, an expert group recommended this test for inclusion into the international OECD (Organization for the Economic Cooperation and Development) Test Guidelines for the Testing of Chemicals. In May 2002 the cell culture test was approved by the OECD.

Fig. 3: Guinea pig used in phototoxicity testing at a French contract laboratory in 2000 (One Voice).

8.6.2 Example No. 2: HET-CAM Test/Draize Test

So far it has only been possible to replace one part of the historic Draize eye irritation test (Huggins, Chapters 2, 3.1), even though it was already early on that this animal experiment came to the centre of the public discussions due to the extreme suffering of the animals. It was not least this public pressure that motivated researchers to look for alternatives to this animal test. For the evaluation of irritating effects to mucous membranes and the eye, increasing concentrations of the test substance are applied into the rabbits' eyes and the resulting effects recorded by subjective evaluation. The damage to the eyes can extend from redness over corrosions to complete blindness of the eye. The so-called HET-CAM test (HET-CAM: Hen's Egg Test – Chorion Allantoic Membrane) (Huggins, Chapter 2.2) has been officially accepted in Germany for the evaluation of highly irritating substances and in France for the evaluation of finished cosmetic products. In this test method, the substance is applied to the egg membrane of incubated hen's eggs. This egg membrane is highly vascularized. The irritating potential of a given substance can be determined with this membrane by measuring resulting tissue damage, such as bleeding, blood vessel dilation or destruction. Substances that are classified to be highly irritating in the HET-CAM need not be tested further on the rabbit eye.

In spite of year long endeavours to obtain a total replacement to the disputed Draize test and in spite of a series of non-animal test methods that are ready for routine application and that are actually in use, such as the Neutral Red Cell Culture Test (Balls et al., 1999; Jones et al., 1999) or the HET-CAM test (Bagley, 1999; Balls et al., 1999), a definitive decision to no longer perform the Draize test is still outstanding. All the same there are a couple of European countries besides Germany in which alternative methods to the Draize eye irritation test have been officially accepted. For the determination of highly irritating substances, the Isolated Rabbit's Eye Test has been accepted in the United Kingdom, the Isolated Chicken's Eye Test in the Netherlands, and the BCOP, a test with isolated bovine eyes, in Belgium.

The protocol of the Draize test was refined, so that the distress the animals have to endure is reduced. However, in the end this is yet another case where those responsible stick to the animal test in spite of the method having been criticised heavily for years, just as was the case with the LD_{50} test. There are a number of scientific publications that show that the Draize eye irritation test gives unreliable and poorly reproducible results (Rieger and Battista, 1964; Weltman et al., 1965; Weil and Scala, 1971; Akademie für Tierschutz, 1989).

The problem is not only that the evaluation of the test results of the Draize test is based upon a subjective variable interpretation of the tissue damage to the rabbit's eye. It is also that the basic anatomical and physiological differences between the eye of the rabbit and the human eye make it impossible to extrapolate the results gained in the Draize test to the situation in humans. This test leads to false predictions of the safety to humans. Therefore, as a final consequence, this test poses a risk to humans. To give some examples, the rabbit has a third eye lid that can influence the contact between the cornea and the test substance. Rabbits produce less eye fluid than humans, so that the substance remains in the rabbit's eye for a longer time and at a higher concentration. Differences can also be found in the capacity of the eye fluid to compensate pH changes, in the tissue structure and in the biochemical characteristics of the cornea. All of these parameters can influence the classification of substances to a great extent and in an unpredictable way, so that one has to question whether the results gained in rabbits are of any value for the estimation of the situation in humans.

8.7 Conclusions

For animal welfarists it is hard to understand and by no means acceptable that years can pass before an alternative method that has undergone a successful scientific validation will be accepted for mandatory use. It is regrettable that there are video documentations showing that even in the year 2000 in a French contract laboratory phototoxicity tests on guinea pigs were performed (Fig. 3), even though at that time the non-animal test method had already been accepted to be scientifically valid and would have been mandatory according to the regulations of Directive 86/609/EEC.

Therefore one has to assume that alternative methods will not be used universally as long as they are not legally requested. It is to be hoped that the procedure of official acceptance of alternative methods will be sped up for future alternative methods now that the first methods have overcome this bureaucratic hurdle.

It remains unchanged that the endeavours are directed at comparing the results of the newly developed *in vitro* test methods to the results of the animal test and at judging them accordingly. However this is an attempt that is due to fail if the starting point itself is unreliable and not reproducible. Gettings et al. (1991) came to the conclusion that one of the reasons that the validation studies for replacement tests to the Draize test failed was the fact that insufficient reliable and reproducible data from the animal test was available. There are two lessons to be learned from this: First, it is entirely impossible to find a good alternative by comparing it to a bad animal test. But secondly and more importantly, citizens and those responsible should question whether it is satisfactory that the estimation of the eye irritating potential of a given substance is performed with a method that is of little relevance to humans.

8.8 The 3Rs in the area of the development of pharmaceuticals

A large number of methods and strategies for the reduction of animal experiments is taken up in an area where official acceptance is not necessary, such as the area of screening for new pharmaceuticals. The lack of requirement of official acceptance is one explanation for the reduction of laboratory animal use in this area. Additionally, in industry cost-benefit-calculations are an important criterion for the decision to try out new possibilities. The use of non-animal test methods, such as computer modelling (for example Computer Assisted Drug Development), biochemical meth-

ods, cell cultures or isolated organs, makes it possible to screen a large number of substances quickly, economically and effectively for their possible wanted and also unwanted effects.

However it is also in this area that the belief in animal experiments is still much too strong. The pharmaceutical industry is of the opinion that the multiple interactions of a substance with the different organs, the components of the endocrine system, etc., can only be evaluated in a complex living organism. However due to technical and scientific progress, it has become possible to produce highly specific cells that express the desired metabolic capacity with the help of gene technology, to cultivate three-dimensional tissues with high functional specificity or to obtain testing material for multiple use with the help of stem cells. Once again, one should not expect that it would be possible to replace an individual animal test with one or two non-animal test methods. Instead, a large number of these methods must be combined to a meaningful testing strategy – for example tailored to the respective product or intended use of the substances to be tested, based upon the knowledge on the biochemical mechanism of the deficiency to be treated and of the substance to be tested.

8.9 The 3Rs in the area of basic research

What about the so-called area of basic research? Of course scientists engaged in the area of basic research are also involved in the development of new test methods. However it has to be stated that – contrary to the scientists that work in industry – in general, scientists that are engaged in research in academia do not do this with the aim of making a contribution to the replacement of animal tests. It is still possible to come across a university researcher who, when asked about the 3Rs, does not even know what this term stands for. Oftentimes the small number of researchers in academia who actually were looking for new non-animal test methods were the ones who pointed out to the other scientists that the method they were developing could possibly be used to replace an animal test or that it was already doing so.

Apart from few exceptions, it is also in basic research that the animal experiment remains the standard method to achieve a goal in the established scientific areas. This is confirmed by the official statistics on animal use that show a surprising constancy in this area over the years. Lately, the numbers have even begun to rise. When evaluating applications for animal experiments to give advice to the German licensing authorities, members of the advisory committees requested by the German Animal Welfare Act again and again are confronted with arguments such as "this study can only be done in a live animal because cells don't suffer from a cold, or because cells don't have a blood circulation", etc. Cell culture methods and other non-animal methods are still seen as pre-screens or as methods to be performed parallel to the animal experiment in order to gain additional information. Such trains of thought culminate in researchers wanting to test the alternative method in the animal experiment, as is shown by the argument of a brain researcher who said: "Before we can use a non-invasive test method, we have to evaluate its results in animal experiments."

Experts believe that the increasing numbers of animals used in basic research are caused by their increased use in the area of gene technology. Many scientists believe or at least contend that the use of transgenic animals makes a contribution to the refinement and reduction of animal tests. They say that, for example, animal models for human genetic diseases could be tailor-made, and that therefore less animals would have to be used in the research studies, or that special transgenic animal lines are more sensitive to certain toxicological endpoints, or that certain disease symptoms could possibly be examined in very early stages in transgenic animals. This point of view should be questioned, first for the very general reason that it is unclear, if genetically manipulated "animal models" are at all adequate to obtain useful information on human diseases, which oftentimes are caused by a multitude of factors. And secondly, when evaluating the true cost of the animal experiment with transgenic animals, the following items have to be taken into account in addition to the estimation of the distress to the animals due to the experimental procedure itself:

- To a large degree, the technologies currently available for the production of transgenic animals are unpredictable and uncontrollable. They do not permit the inclusion of specific genes at specific locations with high efficiency, not to mention their ending up being expressed in the phenotype of the animal.
- The establishment and breeding of a transgenic animal line requires extremely high numbers of animals.
- Additional distress evolves from the housing of the animals, which is mostly specific-pathogen-free, the transportation (transgenic animals oftentimes are bred in separate institutions) and the lack of individual care of the animals, which may be necessary when the animals develop symptoms of the disease or other deficiencies.
- Additional distress is caused when the transgenicity of the animals is evaluated and their phenotype is defined.
- Pain, suffering and damage are inflicted upon transgenic animals that are used as disease models when the respective symptoms develop.
- Oftentimes the genetic manipulation leads to additional deficiencies in the animals.

When all of these factors are considered in a cost-benefit-analysis, it becomes evident that transgenic animals cannot make a contribution to the 3Rs (Salomon et al., 2001). Additionally it is true that the new technology evokes new research needs and there are prognoses that the numbers of animals used will increase further due to research in the area of gene technology. In the United Kingdom, the number of transgenic animals used in experiments increased by 1106% between 1990 and 2000.

8.10 The 3Rs principle in students' education and in ongoing education

Animal experiments are not only performed with the aim of answering unresolved scientific problems or to meet legal requirements, but also with the aim of demonstrating already known facts to students of biology, medicine or

veterinary medicine. From the point of view of animal welfare this is an area where only a total replacement - that is a complete renunciation of using animals killed for that purpose - can be considered acceptable.

In spite of this, it is also in the area of the students'education that the reduction and complete replacement of animal experiments proceeds very slowly, even though a vast number of methods already exist for this purpose. A large number of documentations show impressively that a large diversity of methods are available – from computer simulations to painless self-experiments or video material to plastic models – that can be used to teach the relevant subjects (see for example Akademie für Tierschutz, 1995; Zinko et al., 1997).

The application of such methods is impeded by information deficiencies and ignorance of the responsible teachers as well as by technological and financial obstacles. A further obstacle exists in Germany. That is the fundamental right of freedom of teaching laid down in Article 5, Paragraph III of the Constitution. Lately, court decisions have confirmed that this basic right does not only imply the freedom to choose the subjects to be taught, but also the freedom to determine the method with which to teach the subject. Since this fundamental right is valid without legal reservation, in Germany the decision on whether to choose a didactic method with or without the killing of an animal could be made only by the university teacher himself. This legal situation has changed in the summer of 2002, when animal welfare was included in the German Constitution as national objective. However the legal implications therefrom in practice remain to be shown.

There is one very important argument that speaks in favour of the use of non-animal methods in students' education: The methods that the students become acquainted with during their education will be the ones they will use once they are engaged in research themselves. Only if students learn scientific non-animal methods early on, they will be familiar with them later on and be able to apply them adequately. However, universities that offer classes on the 3Rs are the exception. Unfortunately, adequate legal demands calling for such measures can be found neither in the German Animal Welfare Act nor in the European Directive on the Protection of Laboratory Animals. Deficiencies also exist in the training and further education of those persons who are in charge of the surveillance of the fulfilment of the 3Rs in research. On the other hand, from the first semester on, the experimenters of tomorrow learn that animals are available as arbitrary research objects and that one does not necessarily have to strive for alternatives. The concept only to use animals in research if this is truly inevitable for a specific purpose and there are truly no alternatives is already being counteracted in education by the fact that the use of killed animals is obligatory for almost every biology student regardless of whether their studies are aiming at graduating in zoology, botany or any other specialty area. In this context, the situation is especially confusing for veterinary students. Since they can only graduate with the help of animal use (whereas their colleagues in human medicine are educated without "human experiments"), they must get the impression that the question of whether alternative methods can and must be used is subordinate to other criteria than animal welfare and indispensability.

9 Which conclusions are to be drawn from the presented items in regard to the 3Rs from the point of view of animal welfare?

1. There are good initiatives beneficial to the animal welfare aspect in all three areas, be it refinement, reduction or replacement, even though the state of the art in the area of replacement, which is especially important for animal welfarists, is still not satisfactory.
2. The legal frame for the implementation of animal welfare in the area of animal experiments has been improved – not least due to the discussions that have surrounded the 3Rs in the last 20 years. In spite of this, the demands of the animal welfare organisations have not been implemented in relevant legal documents and even less in day-to-day practice.
3. Both in research and in education, considerable efforts are still necessary in order to fully achieve the fulfilment of the 3Rs principle and to ensure the development and application of non-animal test methods.
4. When developing and validating non-animal test methods for the replacement of animal experiments that have been used for many decades as standard methods to ensure the safety to the consumers and the environment, it is becoming more and more evident that these animal experiments are unreliable test methods that do not even lead to reproducible results. This alone is not surprising, since animal tests – contrary to alternative methods –were never challenged as to their reproducibility or validity. (What is surprising, however, is that the belief in animal experiments is still firmly established in the minds of many scientists.)
5. The choice of laboratory animal species tends to be made more or less arbitrarily, even though in many cases the results from animal experiments will be used to predict whether a test substance might be harmful or beneficial to humans. This results in severe miscalculations of the potential risk to humans.

What is new is that this critical evaluation of animal experiments is increasingly not only made by animal welfarists or antivivisectionists, but also by scientists who are established in the scientific community. This is shown for example by the fact that the abnormal toxicity test has been removed from a large number of test guidelines. From the point of view of animal welfare, it is to be welcomed that the long-lasting criticism of animal experiments is increasingly backed up by research activities in the 3Rs and that again and again some researcher is surprised that a method that he considered to be reliable turns out to be totally unreliable. On the other hand, disbelieving astonishment is evoked in animal welfarists by the fact that this recognition does not spread more widely and above all, hardly leads to consequences. In-

stead, the vast majority of researchers sticks to bad animal experiments and rejects alternative methods that might be better. What thus remains is the belief in the animal experiment as an indisputable rule. This criticism is directed above all towards those who end up responsible for the acceptance of the new methods, that is the representatives of authorities whose decisions are taken over by politicians. However new methods will not receive the opportunity they deserve if they continue to be measured against the results from animal experiments without further thinking and in addition have to overcome the hurdles of validation and acceptance that no animal experiment ever had to overcome.

10 Which is the way forward?

Animal welfarists have a vision of a world without animal experiments. Research in the 3Rs is thus "first aid" for animals and educational program for scientists and authorities underway to better methods. This at least is true for the first two Rs, for refinement and reduction.

Animal welfarists want a consequent turning away from animal experiments. This goal can only be achieved by getting away from the still existing request for a one-to-one replacement that stands in the way to success. Scientific questions must be formulated so that they can be answered with non-animal tests alone. Above all it is the duty of basic researchers to concentrate on developing new non-animal methods for testing and research purposes instead of continuing to produce new animal suffering when looking for new starting-points for research in gene technology and stem cell research.

Toxicology has to get away from the old strategies for safety testing, in which the results from animal tests are tick boxed one after another, oftentimes disregarding the question of whether they will result in meaningful information at all. Instead, intelligent strategies must come into force that are limited to relevant questions and these must be answered with new, reliable methods. This issue also entails questioning the need for a new product before allowing it to be put on the market, because every new product will carry an ultimate risk no matter how good the testing methods applied were.

Much remains to be done. However promising first steps are being tried out, even though they are far too cautious, that bring animal welfarists closer to the vision of a world without animal experiments. This is taking place when animal experiments with which relevant information cannot be collected are removed from legally binding documents. One of the examples for this is the abnormal toxicity test that is required in the European Pharmacopoeia as a testing method for the batch testing of vaccines and sera to determine possible toxic impurities. The best replacement for this test is its removal without substitution. After years of efforts by engaged scientists and animal welfarists, this test was removed from many test guidelines in 1995.

The German Federal Ministry for Education and Research has changed its funding program "Replacement methods to animal experiments" so that now also workshops can be held that serve "to evaluate the existing reduction potential in specific areas of research or the development of recommendations for action". This is a further development of the funding program, with the intention of achieving a better involvement of certain areas of basic research in 3R directed research.

The funding of research in the area of the 3Rs is not restricted to large national or international funding programs. Private organisations or foundations also provide research funding. Even if their budget is restricted, the funding of low-cost projects can be very efficient in pursuing the goal of replacing, reducing and refining animal experiments. Such low-budget funding projects should entail promoting the acceptance of and knowledge on alternative methods, for example when a scientist is granted funding to attend a cell culture class that will enable him to use the cell culture test method instead of the previously established animal test method for his further research. Another example is to grant an initial sum sufficient to get a promising research project started, that – once the first results are available - has a chance to receive further financial contributions by a larger funding program. Small projects that deal with concise, precisely defined areas of research (for example the development of *in vitro* methods to keep parasites for research purposes to replace the use of live intermediate hosts) should also be funded since the positive outcome of such projects will lead to a quick replacement of laboratory animal use.

On the international level there are endeavours to combine national platforms to a strong lobby for alternative methods, the European Consensus Platform for Alternative Methods (ecopa). The reason for this is that many animal tests are included in documents that are legally binding throughout the European Union and these are based upon OECD Test Guidelines. If one strives to remove animal tests that are proven to be outdated from such regulations or to include new alternative test methods that make use of no or at least fewer animals, this has to be achieved on the European and international level. In order for the project of ecopa to succeed, all national platforms need a common foundation. All four main interest groups – authorities, industry, academia and animal welfare – must sit at one table in the national platform. Only then will it be possible to ensure a transparent, democratic and efficient procedure on the international level. Ecopa has only recently been founded. It is to be hoped that it will develop in the right manner so that it can truly fulfil its goal to promote alternative methods.

The discussion surrounding the new EU chemicals policy is also promising. For the first time it is foreseen that for low production volume chemicals in general only data from non-animal test methods are to be requested for registration. The European Centre for the Validation of Alternative Methods has presented a testing strategy with which it is possible to collect data for the classification of chemicals making use of non-animal test methods. This is an example for a strategic procedure instead of a tick-boxing of inflexible regimes. In spite of all this, many obstacles still

remain, above all the obstacle that many of those responsible still believe that safety testing without animal experiments is not possible.

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